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Improving CO_2 fixation efficiency by optimizing *Chlorella* PY-ZU1 culture conditions in sequential bioreactors

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HIGHLIGHTS

- Nutrients of N, P and Mg were optimized for *Chlorella* PY-ZU1 under 15% CO_2.
- Biomass had a 1.25-fold increase (from 2.41 to 5.42 g L^{-1}) by culture optimization.
- Peak growth rate of microalgae increased by 99% through enhancing light intensity.
- Peak CO_2 fixation efficiency in a sequential bioreactor was 85.6% under 15% CO_2.

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ABSTRACT

To fix CO_2 emissions efficiently from flue gas of coal-fired power plants, the culture medium, light intensity and bioreactors were comprehensively optimized in the process of CO_2 fixation by *Chlorella* PY-ZU1. To make up for relative insufficiency of nutrients (except for the carbon source) resulting from continuous bubbling of 15% CO_2, three chemicals were added into the culture to optimize the molar ratios of nitrogen to carbon, phosphorus to carbon, and magnesium to carbon in culture from 0.17 to 0.69, from 0.093 to 0.096, and from 0.018 to 0.030, respectively. Such adjustments led to a 1.25-fold increase in biomass (from 2.41 to 5.42 g L^{-1}). By enhancing light intensity from 4500 to 6000 lux, the peak growth rate of *Chlorella* PY-ZU1 increased by 99% and reached to 0.95 g L^{-1} day^{-1}. Use of a multi-stage sequential bioreactor notably improved the peak CO_2 fixation efficiency to 85.6%.

1. Introduction

Billions of tons of CO_2 was released into the atmosphere every year and aggravated the global warming. Thus, the reduction of large CO_2 emissions via an efficient and economic method is urgently required. A promising technology is the biological capture of CO_2 using microalgae. These microorganisms can fix CO_2 using solar energy with efficiency (2–10%) ten times greater than terrestrial plants (<1%), and have a higher growth rate (1- to 3-fold increases in biomass per day). Also the use of microalgae is a highly efficient approach for converting CO_2 into biomass. Moreover, the CO_2 capture process using microalgae has the following advantages: (i) being an environmental sustainable method that can be connected to urban and industrial sewage cleaning (Carpenter et al., 1995; Jiang et al., 2011); (ii) co-producing high added value materials based on biomass, such as biofuel and biogas (Cheng et al., 2012; Chisti, 2007; Clarens et al., 2010; Yoo et al., 2010). Generally, the characteristics of flue gas in coal-fired power plants include large flow rates, high CO_2 content (~15%), and the presence of toxic compounds, such as SO_2, NO_x and fine particles. That made it hard to fix such large CO_2 emissions from power plants using wild microalgae because of the toxic compounds, and the need of gargantuan farming areas. As such, improvement of fixation efficiency of continuous and high concentrations of CO_2 by microalgae is critical.

CO_2 fixation efficiency by microalgae is dependent on microalgae species (Toledo-Cervantes et al., 2013), nutrients ratio, light intensity, temperature, pH, CO_2 concentration and flow rate, and photo-bioreactor type, in general, the CO_2 fixation efficiency is directly proportional to the microalgae growth rate (González López et al., 2009). Ramanan et al. (2010) has demonstrated an increase in CO_2 sequestration efficiency by maneuvering chemically aided biological sequestration of CO_2. *Chlorella* sp. and *Spirulina platensis* showed 46% and 39% max fixation efficiency, respectively, at input CO_2 concentration of 10%. de Morais and Costa (2007) have reported that CO_2 fixation rate could be sharply increased in a three-stage serial tubular photo-bioreactor. The Maximum daily carbon dioxide bio-fixation by *Spirulina* sp. was 45.61%, at input CO_2 concentration of 12%. Chiu et al. (2008) replaced half of volume of the culture broth with fresh medium every day to enhance

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high growth rate of *Chlorella* sp. and CO_2 reduction. Efficiency of CO_2 removal by *Chlorella* sp. was 16%, at input CO_2 concentration of 15%.

In recent reports, the efficiency of CO_2 fixation from flue gas by microalgae was found to be generally less than 50%. Methods by which to improve fixation efficiency of continuous and high concentrations of CO_2 are vital technical difficulties. Many previous studies related to CO_2 fixation by microalgae have focused on screening microalgae and strains that can tolerant high concentrations of CO_2 (Sung et al., 1999; Yue and Chen, 2005), and testing their CO_2 fixation ability with culture conditions obtained under air or low concentrations of CO_2 (Ramanan et al., 2010; Yoo et al., 2010), for example the culture medium, such as SE, BG11 medium (Yoo et al., 2010), and *f*/2 medium (Cheng et al., 2006; Chiu et al., 2008). However, these previous studies neglected the changes caused by continuous bubbling of high concentration of CO_2 on the optimum molar ratios of nutrients, light intensity, and empty bed residence time (EBRT) of the bioreactor that microalgae needed. For lacking of knowledge on optimum microalgae culture conditions aimed at high concentration of CO_2, increasing CO_2 fixation efficiency is challenging.

Therefore, in our study, *Chlorella* PY-ZU1, a species that obtained by γ irradiation and high concentrations of CO_2 domestication from *Chlorella pyrenoidosa*, was used. The culture medium, light intensity and EBRT of bioreactor were comprehensively optimized with continuous bubbling of 15% CO_2 (the concentration of CO_2 from flue gas in most coal-fired power plants), which made a breakthrough increase on the peak CO_2 fixation efficiency (85.6%), as well as microalgae biomass productivity.

2. Methods

2.1. Strains and media

Chlorella PY-ZU1 used in this study was a strain that obtained by γ irradiation and high concentrations of CO_2 domestication from *C. pyrenoidosa*. In our previous study, *Chlorella* PY-ZU1 showed a 32.7% of peak CO_2 fixation efficiency under 15% CO_2 (Cheng et al., 2013). The cells of *Chlorella* PY-ZU1 were maintained in Brostol's solution (also known as soil extract, SE) (Cheng et al., 2013), containing 0.25 g of $NaNO_3$, 0.075 g of $K_2HPO_4{\cdot}3H_2O$, 0.075 g of $MgSO_4{\cdot}7H_2O$, 0.025 g of $CaCl_2{\cdot}2H_2O$, 0.175 g of KH_2PO_4, 0.025 g of NaCl, 40 mL of soil extract, 0.005 g of $FeCl_3{\cdot}6H_2O$, 1 mL of Fe-EDTA, and 1 mL of A_5 solution in 958 mL of de-ionized water.

2.2. Optimization of culture medium and light intensity for microalgae under 15% CO_2

Optimization experiments for the culture medium and light intensity were performed in an artificial greenhouse at 27 °C. 300 mL culture broth with 30 mL of *Chlorella* PY-ZU1 pre-culture was inoculated into a column bioreactor (BR) (160 mm × Φ56 mm, 300 mL working volume), which has a long steel pipe (180 mm × Φ3 mm) to the bottom for 15% CO_2 gas bubbling in at a rate of 30 mL min^{-1}. The initial pH was adjusted to 6.5 with 0.1 M HCl and 0.1 M NaOH. During incubation, continuous light of 4500 lux at the surface of BR was supplied by four cool white lights combined with two plant lights (Philips, TLD 36 W) that were fixed above the BR. The initial concentrations of $NaNO_3$, $MgSO_4$, and K_2HPO_4 (but not KH_2PO_4 for it is more acid) in the SE medium were optimized. To determine the optimal initial nitrate concentration for biomass productivity under 15% CO_2, *Chlorella* PY-ZU1 was cultivated with SE medium containing 3, 6, 9, 12, and 18 mM $NaNO_3$, respectively. To determine the optimal initial concentrations of magnesium and phosphate for biomass productivity, 0.305, 0.61, 0.915, 1.22, and 1.525 mM $MgSO_4$ and 0.329, 0.493, 0.658, 0.822, and 0.987 mM K_2HPO_4, respectively, were supplemented into the medium with 12 mM nitrate. The optimal medium aimed at 15% CO_2 was obtained and abbreviated as SE*. This medium contains 12 mM $NaNO_3$, 0.61 mM $MgSO_4$, 0.658 mM K_2HPO_4, with all other conditions same to those in the SE medium. During cultivation, nitrate concentrations were measured by the cadmium reduction method (Patton et al., 2002). Chlorophyll were extracted out from microalgae cells by soaking in DMSO/80% acetone (1/2,V/V) and then measured (Lorenzen, 1967).

In order to further improve biomass productivity under 15% CO_2, more light (6000 lux) was supplied for *Chlorella* PY-ZU1 cultivation, which was expected to improve the microalgae growth rate and shorten its growth cycle.

2.3. Experiments of CO_2 fixation in sequential bioreactors under 15% CO_2

All the experiments were performed using SE* medium in the artificial greenhouse. In each BR (300 mL of working volume), 30 mL of *Chlorella* PY-ZU1 pre-culture was inoculated into 270 mL SE* medium. The bioreactors were connected in series to form sequential bioreactor. Exactly 30 mL min^{-1} of 15% CO_2 was continuously aerated into the inlet of the first bioreactor in the several-stage sequential system, flowed out from the outlet of the first bioreactor to the next bioreactor and so on. Lastly, the gas was flowed out into an infrared online CO_2 analyzer (SERVOMEX4100, UK). It realized CO_2 multi-utilization and fixation. When aerated with 30 mL min^{-1} of 15% CO_2, the empty bed residence time (EBRT) of one bioreactor (300 mL of working volume) was 10 min, while the EBRT of a 9-stage sequential bioreactor (2700 mL of working volume) and 14-stage sequential bioreactor (4200 mL of working volume) were 90 and 140 min, respectively.

2.4. Analysis of biomass (dry weight) and growth rate of Chlorella

During cultivation, 10 mL of the samples was dewatered by centrifugation (Beckman Avanti J26-XP, USA) at 8500 rpm for 10 min, and dried at 70 °C for 24 h to obtain the dried biomass weight. Biomass concentration (g L^{-1}) was calculated from the microalgae dry weight produced per liter. The growth rate (AGR, g L^{-1} day^{-1}) was calculated using Eq. (1):

$$\mathrm{AGR} = \frac{M_1 - M_2}{t_1 - t_2} \tag{1}$$

where M_1 is the biomass concentration at time t_1 and M_2 is the biomass concentration at time t_2.

2.5. Calculation of DIC and CO_2 fixation efficiency

Dissolved inorganic carbon (DIC) in the culture was calculated according to the CO_2 dissolved process (Matsuda et al., 2001) as follows:

$$CO_2(g) + H_2O \leftrightarrow H_2CO_3(g) \quad K_H = \frac{[H_2CO_3]}{P_{CO_2}}$$

$$H_2CO_3(g) \leftrightarrow H^+ + HCO_3^-(g) \quad K_{a1} = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

During cultivation, the pH of culture was 5.5–7, thus:

$$[\mathrm{DIC}] = [H_2CO_3] + [HCO_3^-] = K_H \times P_{CO_2} \times \left(1 + \frac{K_{a1}}{H^+}\right) \tag{2}$$

At a constant temperature of 27 °C, $K_H = 3.2 \times 10^{-2}$ M/atm and $K_{a1} = 4.3 \times 10^{-7}$ M (Millero et al., 2006).

When 15% CO_2 was aerated into the SE medium with *Chlorella* PY-ZU1 in the BR, the CO_2 concentration of outlet was 14.24% while the pH of the culture remained constant at 6.8. Thus, $[H^+] = 10^{-pH} = 1.58 \times 10^{-7}$, $P_{CO2} = \text{mean}\ (P_{CO2input}, P_{CO2output}) = 0.1462$ atm.

According to Eq. (2):

$$[DIC]_{SE} = 3.2 \times 10^{-2}\,M/atm \times 0.1462\,atm \times \left(1 + \frac{4.3 \times 10^{-7}}{1.58 \times 10^{-7}}\right) = 17.41\,mM.$$

The influent and effluent CO_2 concentrations were monitored online by a CO_2 analyzer (SERVOMEX4100, UK), and the gas flow rate was controlled and measured by a Mass Flow meter (SevenstarCS200, China). The amount of CO_2 reduction was the carbon dioxide difference between influent and effluent, and CO_2 removal efficiency calculated according to flowing equation:

$$CO_2\ \text{removal efficiency}\,(\%) = \left(1 - \frac{CO_{2output}}{CO_{2input}}\right) \times 100\% \quad (3)$$

where $CO_{2output}$ = influent CO_2 concentration × influent flow rate; CO_{2input} = effluent CO_2 concentration × effluent flow rate.

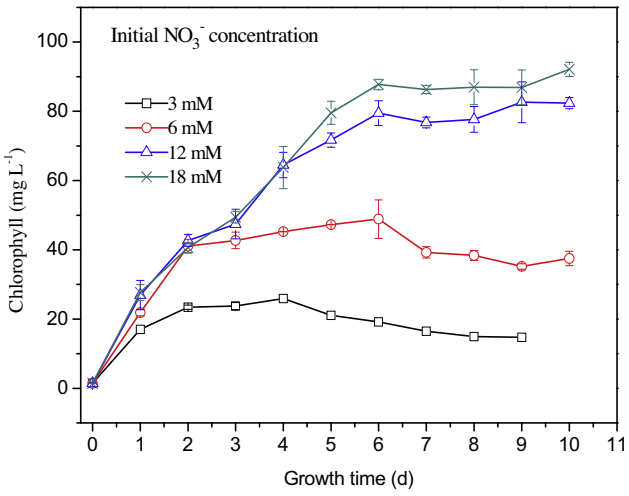
3. Results and discussion

3.1. Optimization of the culture medium to improve Chlorella PY-ZU1 biomass productivity under 15% CO_2

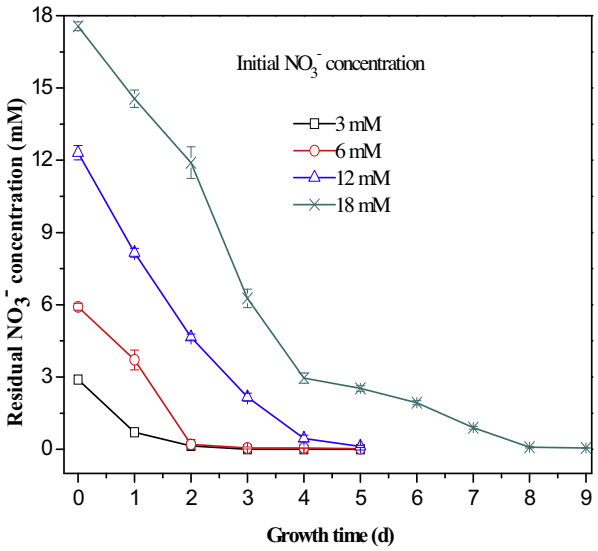
Continuous bubbling of 15% CO_2 produced 17.41 mM DIC in the SE culture. However, the initial contents of three main elements (nitrogen [N], phosphorous [P], and magnesium [Mg]) in the SE medium were 3, 1.615, and 0.305 mM, respectively, and calculated as follows: $[N]_{SE} = [NaNO_3] = 3$ mM, $[P]_{SE} = [K_2HPO_4] + [KH_2PO_4] = 1.615$ mM, $[Mg]_{SE} = [MgSO_4] = 0.305$ mM. In this study, the molar ratios of nitrogen to carbon (N/C), phosphorous to carbon (P/C), and magnesium to carbon (Mg/C) were respectively determined as the ratios of the initial [N], [P] and [Mg] in the culture medium to the total DIC content (sum of DIC produced by aeration of CO_2 and initial content in the medium). For there was no carbon source in the medium, the total DIC was the DIC produced by CO_2 dissolved into culture. When continuously bubbled with 15% CO_2, the molar ratio of N/C was 0.71 (N/C = 3/17.41 = 0.71), that of P/C was 0.093 (P/C = 1.615/17.41 = 0.093), and that of Mg/C was 0.018 (Mg/C = 0.305/17.41 = 0.018).

Considering the sustained 17.41 mM DIC in culture, the initial contents of the three nutrient elements were relative deficient, resulting in limitation on microalgae growth and CO_2 fixation. Thereby, more nutrients (not including the carbon source), should be added to the culture to improve biomass productivity.

Nitrogen is an essential element for chlorophyll and protein synthesis. Increased molar ratio of nitrogen to carbon under 15% CO_2 could promote chlorophyll synthesis and transfer more energy for CO_2 fixation. In addition, it could improve the protein contents of cell. Which may lead to acceleration of photosynthesis (Kim et al., 2012). Fig. 1 showed that the maximum content of chlorophyll was increased sharply from 23 to 96 mg L^{-1} with the initial nitrate concentration increased from 3 to 18 mM (Fig. 1a), which would improve the microalgae biomass productivity. After enrichment of the microalgae with 3 mM nitrate on day 0, a rapid increase in chlorophyll concentration and decline in nitrate concentration were observed (Fig. 1b). About 75% of the initial nitrate was consumed by day 1 during synthesis of 19 mg L^{-1} chlorophyll. Chlorophyll production reached a peak value of 23 mg L^{-1} by day 2, during which the nitrate had been completely taken up. These results indicate that a 3 mM initial nitrate concentration in the SE medium is relatively deficient and significantly limits *Chlorella* PY-ZU1 photosynthesis. Increasing the initial nitrate concentration to 6 and 12 mM could extend the time of nitrate consumption by up to 3 and 5 days, respectively, with peak chlorophyll contents of 48 and 82 mg L^{-1}. It had reported that chlorophyll synthesis is almost directly proportional to the initial nitrate content in the culture (Edwards et al., 2003). For *Chlorella* PY-ZU1 in this study, chlorophyll synthesis linearly increased from 23 to 82 mg L^{-1} in response to an increase in the initial nitrate concentration from 3 to 12 mM. That is 6.73 mg chlorophyll produced with 1 mM nitrate added. However, excessively high nitrate concentration (18 mM) had little effect on chlorophyll synthesis (96 mg L^{-1}), although the nitrate consumption time extended to day 8, which is attributed to increased rates of chlorophyll synthesis. Excessive production of chlorophyll, as evidenced by the dark green coloration of the culture, could limit the effective depth penetration of light.



(a) Chlorophyll synthesis with different initial nitrate



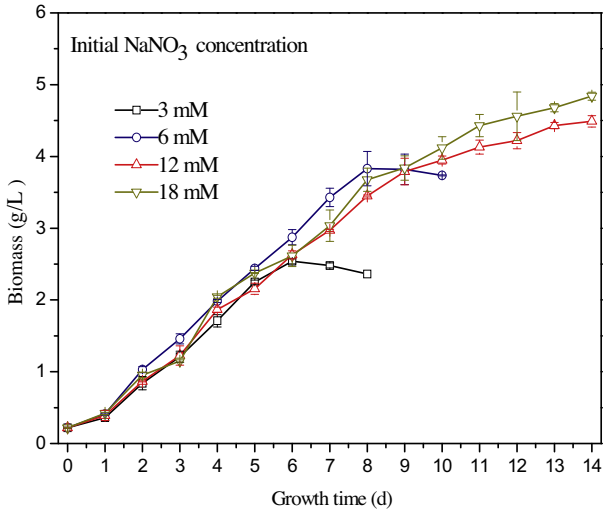
(b) Consumption curves of different initial nitrate

Fig. 1. Chlorophyll synthesis of *Chlorella* PY-ZU1 and nitrate consumption under 15% (v/v) CO_2.

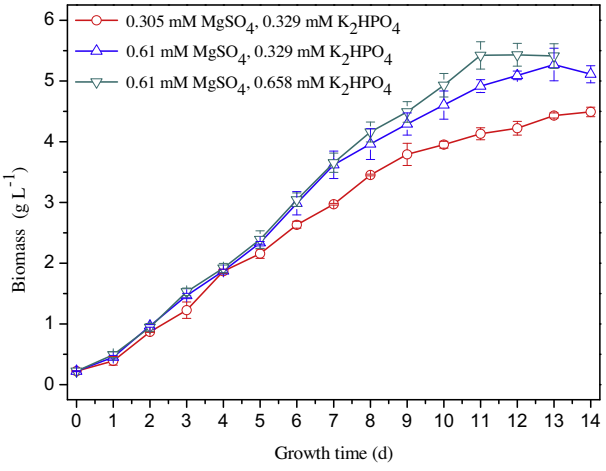
The maximum biomass concentration of *Chlorella* PY-ZU1 increased to 4.84 g L^{-1} with increasing initial nitrate concentration from 3 to 12 mM (Fig. 2a). However, the growth cycle (the time from the adaptation period to attenuation phase) extended from 6 to 14 days. When cultivated with 3 mM nitrate, the peak growth rate of *Chlorella* PY-ZU1 reached 0.48 g L^{-1} day^{-1} on day 2, and maximum biomass concentration was 2.41 g L^{-1} on day 6. At 6 mM initial nitrate, the maximum biomass concentration increased by 54.77% to 3.73 g L^{-1} on day 8. At 12 mM initial nitrate, the maximum biomass concentration was 4.49 g L^{-1} (0.86-fold increases) on day 14. Further increases in initial nitrate concentration (18 mM) brought about only a slight increase in maximum biomass concentration (4.84 g L^{-1} by day 14). For example, only a 7.8% increase in biomass production was obtained with a 50% increase in initial nitrate concentration (from 12 to 18 mM). These results are attributed to the significant increase in chlorophyll synthesis at higher nitrate levels, as evidence by the increase in dark green coloration of the cultures. Excess chlorophyll in the culture blocks the effective penetration of light and leads to great losses in absorption light intensity by cells (Pilon et al., 2011). In consequence, excess nitrate has a little impact on biomass but significantly affects and increases the length of the microalgae growth cycle. Taking into consideration of the energy utilization efficiency and economic costs involved in cultivation, 12 mM was determined as the most suitable initial nitrate concentration for *Chlorella* PY-ZU1 growth and carbon fixation under 15% CO_2.

It has reported that nutrients such as nitrate and phosphorous can stimulate biomass production but to different extents (White and Payne, 1977). The maximum biomass of microalgae increased from 4.43 to 5.27 g L^{-1} (0.20-fold increases) at magnesium sulfate concentrations ranging from 0.315 to 0.610 mM but decreased at magnesium sulfate concentrations above 0.925 mM because of toxicity of sulfate. Similarly, certain levels of phosphorous increase could inhibit microalgae growth. When the initial K_2HPO_4 concentration in the medium was increased from 0.329 to 0.658 mM, the maximum biomass slightly increased from 5.27 to 5.42 g L^{-1} (0.03-fold increases) on day 10. However, at the initial K_2HPO_4 concentrations above 0.658 mM, there was no effect on chlorophyll synthesis (Menéndez et al., 2002) but an increases in cell osmotic pressure was observed. These effects could cause adverse impacts on the growth of microalgae.

In summary, the optimized SE medium (SE*) was obtained. This medium contains 12 mM $NaNO_3$, 0.61 mM $MgSO_4$, 0.658 mM K_2HPO_4, with all other conditions same with those in the SE

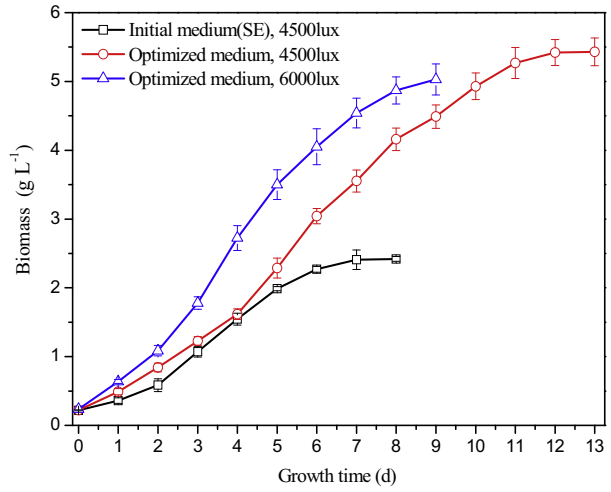


(a) Effect of initial nitrate on biomass production of *Chlorella* PY-ZU1

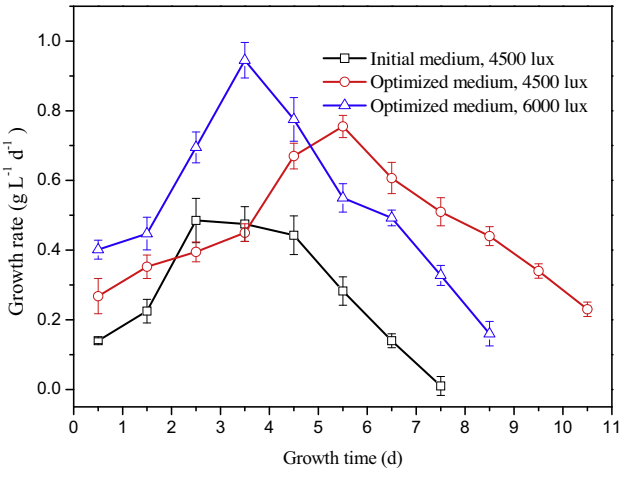


(b) Effects of initial phosphate and magnesium concentrations on biomass production.

Fig. 2. Effects of initial growth media on biomass production of *Chlorella* PY-ZU1 under 15% (v/v) CO_2.



(a) Biomass concentration of Chlorella PY-ZU1



(b) Growth rate of Chlorella PY-ZU1

Fig. 3. Biomass concentration and growth rate of *Chlorella* PY-ZU1 under 15% (v/v) CO_2 with optimized growth media and light intensity.

medium. The initial [N], [P], and [Mg] of SE* medium calculated as follows were 12, 1.944, 0.610 mM, respectively. $[N]_{SE*} = [NaNO_3] = 12$ mM; $[P]_{SE*} = [K_2HPO_4] + [KH_2PO_4] = 1.944$ mM; $[Mg]_{SE*} = [MgSO_4] = 0.610$ mM. When 15% CO_2 was aerated into the SE* medium with *Chlorella* PY-ZU1 in the BR, the CO_2 concentration of outlet was13.81%, the pH of the culture remained constant at 6.9. According to Eq. (2), DIC in the SE* culture was 20.35 mM calculated as follows: $[DIC]_{SE*} = 3.2 \times 10^{-2}$ M/atm $\times 0.1441$ atm $\times \left(1 + \frac{4.3\times10^{-7}}{1.26\times10^{-7}}\right) = 20.35$ mM. The molar ratio of N/C was 0.59 (N/C = 12/20.35 = 0.59), that of P/C was 0.096 (P/C = 1.944/20.35 = 0.096), and that of Mg/C was 0.030 (Mg/C = 0.61/20.35 = 0.030), respectively. Thus, for *Chlorella* PY-ZU1 cultivation under 15% CO_2, the optimal molar ratios of N/C, P/C, Mg/C of the culture were 0.59, 0.096, and 0.030, respectively.

3.2. Enhancement of light intensity to improve Chlorella PY-ZU1 growth rate under 15% CO_2

Chlorella PY-ZU1 cultivated with SE* medium showed linear increases in cell concentration. This linear increase indicates that illumination would have been the growth-limiting factor in the culture (Sung et al., 1999). Fig. 3 shows that under 4500 lux light intensity, the peak growth rate and maximum biomass concentration of *Chlorella* PY-ZU1 cultured with SE* medium increased to 0.76 g L^{-1} day^{-1} (0.48 g L^{-1} day^{-1} with SE medium), and 5.42 g L^{-1} (2.25-fold to that with SE medium), respectively. However, the time of peak growth rate was delayed to day 5.5 (day 2.5 with SE medium), and the growth cycle was prolonged from 7 to 12 days. When illumination was enhanced to 6000 lux, the light intensity absorbed by the cells for photosynthesis increased, resulting in a 0.95 g L^{-1} day^{-1} of peak growth rate on day 3.5. By increasing the light intensity from 4500 to 6000 lux, the growth cycle of *Chlorella* PY-ZU1 was decreased from 12 to 8 days. This result was consistent with the report that microalgae with higher light intensity needed shorter photoperiod (Yan and Zheng, 2013). And the mean growth rate was significantly accelerated (1.25-fold) under increased illumination.

Anjos et al.(2013) optimized CO_2-mitigation by *Chlorella vulgaris* P12 under different CO_2 concentrations (ranging from 2% to 10%). Results showed that 6.5% was the most appropriate CO_2 concentration for *Chlorella vulgaris* P12, and 0.9 g L^{-1} day^{-1} of the maximum growth rate was obtained under 10 min of EBRT. When *C. pyrenoidosa* was cultivated with SE medium under 10 min of EBRT (Cheng et al., 2013), it also showed that 6% was the most appropriate CO_2 concentration with the maximum growth rate of 0.48 g L^{-1} day^{-1}. However, by optimizing the culture conditions (including growth medium and light intensity), the most appropriate CO_2 concentration for *Chlorella* PY-ZU1 was 10 to 12% (v/v) under 10 min of EBRT, and the maximum growth rate was 0.95 g L^{-1} day^{-1}.

Element analysis results of the dried biomass cultured under optimized conditions were shown in Table 1. The nitrogen content of biomass obtained at 15% CO_2 (2.263%) was smaller than that at air (384 ppm CO_2), which may be due to the abundance of the DIC produced by high levels of CO_2. The amount of carbon dioxide and bicarbonate transporters may decide the carbon transfer rate. It may change the ratios of assimilation of carbon and nitrogen and the cell compositions may be different (Hsueh et al., 2007). In this study, large amounts of carbon dioxide produced by high level of CO_2 bubbling in promoted high molar ratio of C/N (25.58) in biomass. These results indicate that the nitrogen source is inefficient

Table 1
The elemental compositions of *Chlorella* PY-ZU1 dried biomass under 15% (v/v) CO_2 with optimized growth media and light intensity.

Growth conditions	Carbon (%)	Nitrogen (%)	Hydrogen (%)	Oxygen[b] (%)	Carbon/nitrogen (mol/mol)	Biomass formula
SE, air (CO_2 = 384 ppm), 4500 lux	45.478	7.322	6.821	33.129	7.25	$CH_{1.8}O_{0.546}N_{0.138}$
SE, 15% CO_2 [a], 4500 lux	49.268	2.263	7.488	40.981	25.58	$CH_{1.82}O_{0.623}N_{0.039}$
SE*, 15% CO_2, 4500 lux	46.980	3.924	7.063	42.033	13.95	$CH_{1.8}O_{0.671}N_{0.072}$
SE*, 15% CO_2, 6000 lux	47.647	4.211	7.152	40.990	13.20	$CH_{1.8}O_{0.645}N_{0.076}$

SE: SE medium; SE*: optimized SE medium.
[a] The culture was continuously aerated with 15% concentration of CO_2.
[b] The oxygen contents were calculated from mass balance of biomass.

at the condition of high level of CO_2 (15%), and that the initial nitrate level in the medium must be optimized. The C/N of biomass cultured with the optimized medium sharply decreased to 13.95 while nitrogen content of biomass increased to 3.924. The C/N of the biomass obtained with enhancing of light intensity (6000 lux) would slightly decrease to 13.20 but remained higher than that in air (7.25), which may be due to sustained DIC in the culture produced by continuous bubbling with 15% CO_2. Although nitrogen deficient was relieved by addition of more nitrate to the medium, the molar ratio of C/N of the culture with 15% CO_2 remained consistently higher than that of culture aerated with air. In other words, the phenomenon of carbon excess and nitrogen shortage in the culture always occurs but has no effect on the normal growth of microalgae.

3.3. Increasing the EBRT of sequential bioreactor to improve CO_2 fixation efficiency

Chlorella PY-ZU1 was cultivated with the optimized medium aerated with 30 mL min^{-1} of 15% CO_2 to determine its biomass productivity and CO_2 removal ability in a sequential bioreactor. The EBRT of BR, 9-stage sequential bioreactor, and 14-stage sequential bioreactor are 10, 90, and 140 min, respectively. Similar mixing and uniformity of the culture caused by gas bubbles resulted in almost the same biomass productivities for each BR in the multi-stage sequential bioreactor (Table 2). The peak growth rates of microalgae in 9-stage sequential bioreactor and 14-stage sequential bioreactor were 0.88 and 0.83 g L^{-1} day^{-1}, respectively. While their maximum biomass concentrations were 4.39 and 4.74 g L^{-1} on day 8, respectively. These results were almost consistent with those of a single bioreactor, which showed a peak growth rate of 0.95 g L^{-1} day^{-1} and biomass concentration of 4.87 g L^{-1}. Microalgae in the sixth BR of 9-stage sequential bioreactor and in the eighth BR of 14-stage sequential bioreactor, in which the CO_2 concentration was about 10.5% to 12% (v/v) and 10% to 12% (v/v), respectively, showed excellent growth. These findings indicate the most appropriate CO_2 concentration for *Chlorella* PY-ZU1 is to 10% to 12% (v/v). Li et al. (2013) constructed a closed raceway pond by covering a normal open raceway pond with a specially designed transparent cover to prevent supplied CO_2 escaping into atmosphere and thus increase the CO_2 retention time. However, a multi-stage sequential bioreactor was used to increase the CO_2 retention time in our study. In the multi-stage sequential bioreactor, CO_2 was graded utilized and multi-captured by microalgae, and the residence time of CO_2 in algae culture was extended exponentially, thereby resulting in an effective increase in CO_2 fixation efficiency.

The sequential bioreactor was filled with SE* medium and operated for 1 day without microalgae to test for the abiotic removal of CO_2. CO_2 quickly dissolved into the medium in the first 1 h, resulting in a 96% CO_2 removal efficiency (Fig. 4). After 8 h, the effluent CO_2 concentration was nearly equivalent to the influent. That is, CO_2 removal by SE* medium occurred mainly in the first 8 h and should be eliminated in the calculation of CO_2 fixation efficiency

Table 2
Biomass productivity and CO_2 fixation ability of *Chlorella* PY-ZU1with different empty bed residence time (EBRT) under 15% CO_2 with optimized growth condition for 8 days.

EBRT (min)	Biomass production (g L^{-1})	Peak biomass growth rate (g L^{-1} day^{-1})	Peak CO_2 fixation rate (g day^{-1})	Mean CO_2 fixation efficiency (%)
10	4.87	0.95	0.95	7.60
90	4.74	0.88	7.54	50.31
140	4.39	0.83	10.51	70.48

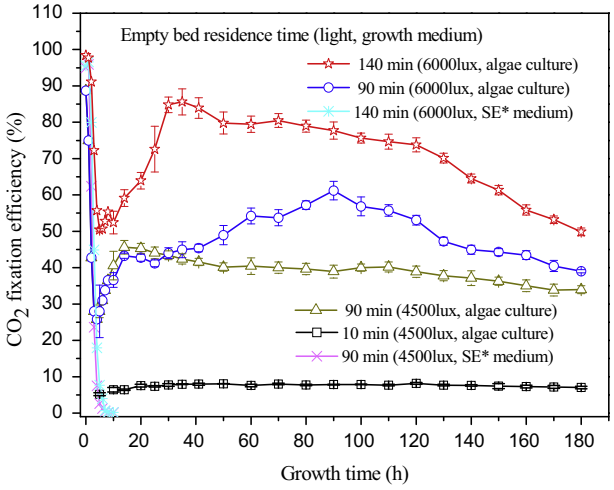


Fig. 4. Effects of empty bed residence time and light intensity on CO_2 fixation efficiency by *Chlorella* PY-ZU1 under 15% CO_2. SE*: optimized SE growth medium.

by microalgae. Under 4500 lux of light intensity, CO_2 fixation efficiency by *Chlorella* PY-ZU1 cultured with a EBRT of 10 min increased from 5% to 8% within the first 2 days, and then stabilized to 7.5–8% within the following 7 days (Fig. 4), and the average CO_2 fixation efficiency was 7.60%. However, when cultured with a 90 min of EBRT, the peak CO_2 fixation efficiency (45%) by microalgae was obtained after 14 h of cultivation, and then decreased slowly to 34% in the next 180 h. When the light intensity was increased to 6000 lux on day 2, the CO_2 fixation efficiency increased to achieve a peak of 61.2% by the 90th hour. Afterward, CO_2 fixation efficiency decreased with decreasing of microalgae growth rate due to nutrients consumption. During the cultivation, the average CO_2 fixation efficiency was 50.31%. When the EBRT was further increased to 140 min, the highest fixation efficiency (85.6%) was observed at the 35th hour in 14-stage sequential bioreactor, and then stabilized to 75–80% during the following 5 days (Fig. 4). The CO_2 fixation efficiency slowly decreased to 45.33% at the 192th hour. The average CO_2 fixation efficiency was 70.48% during the whole experiment period, as shown in Table 2.

The amount of CO_2 fixation showed a linear increase with cultivation time. The peak CO_2 fixation rate (Table 2) was increased from 0.95 g day^{-1} in the BR (10 min of EBRT) to 7.54 g day^{-1} in the 9-stage sequential bioreactor (90 min of EBRT), and then to 10.51 g day^{-1} in 14-stage sequential bioreactor (140 min of EBRT). The total amounts of CO_2 fixation in 9- and 14-stage sequential bioreactors were 6.61- and 9.27-fold to that in BR, respectively, instead of the expected 9- and 14-fold. This result may be due to variations in the concentration of CO_2 in each unit bioreactor of the multi-sequential bioreactors was varied during cultivation. CO_2 concentration decreased in sequence while O_2 concentration increased, which would inhibit photosynthesis of microalgae in downstream bioreactors (Suzuki and Ikawa, 1984), and result in a slight declines in microalgae growth rate and CO_2 fixation efficiency.

4. Conclusion

Optimization of the culture conditions under continuous bubbling of 15% CO_2 was an efficient method to improve biomass productivity and CO_2 fixation ability of microalgae. The maximum biomass concentration and peak growth rate of *Chlorella* PY-ZU1 cultured with optimized medium and light intensity were 5.42 and 0.95 g L^{-1} day^{-1}, respectively. The peak CO_2 fixation efficiency of *Chlorella* PY-ZU1 in 14-stage sequential bioreactor increased to

85.6%. Further efforts should be taken to develop a more efficient CO_2 fixation system composed of microalgae incubation bioreactor, exponential growth bioreactor, and biomass harvesting bioreactor.

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